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# US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY 40121

ANNUAL PROGRESS REPORT, FY 1967

AD819320

RCS MEDDH-288(R1)

30 June 1967



# STATEMENT #2 UNCLASSIFIED

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UNITED STATES ARMY

UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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# HEADQUARTERS US ARMY MEDICAL RESEARCH LABORATORY Fort Knox, Kentucky 40121

# ANNUAL PROGRESS REPORT, FY 1967 RCS MEDDH-288(R1)

30 June 1967

#### FY 1967 Projects:

- 3A013001A91C In-House Laboratory Independent Research
- 3A014501A74D Military Psychophysiological Studies
- 3A014501B71P Basic Research in Support of Military Medicine
- 3A014501B71R Research in Biomedical Sciences
- 3A014501B74C Basic Research in Performance Effectiveness
- 3A025601A819 Army Aviation Medicine
- 3A025601A821 Combat Surgery

#### SUMMARY

The research and development effort at the US Army Medical Research Laboratory, Fort Knox, Kentucky, is devoted to psychological studies of the soldier; laser radiation; methodology relating to the collection, processing, preservation, shipment and transfusion of human blood; the health of laboratory animals; and detoxification of snake venom.

The progress during Fiscal Year 1967 and the current status of the various work units are reported herein.

#### FOREWORD

The work of the Blood Transfusion Division continued to expand, and during the last half of the year, a Blood Donor Center was established in order to carry out the additional mission of supplying quantities of whole blood to the military.

Accelerated laser research shows promise of opening new areas of increasing importance and value to the combat soldier. More intensive work is being directed toward establishment of safety thresholds.

Research involving snake venoms was somewhat reduced during FY 1967, with a part of the effort of the Biochemistry Division being redirected to studies involving blood chemistries.

The Experimental Psychology Division expanded its research in the Skill Analysis Branch to include testing of candidates for rotary wing pilot training. This work is being carried out in conjunction with Phase I training at Fort Wolters, Texas. Continued progress was made in the other research disciplines of this division, with recent developments in the audition field showing promise of considerable military significance.

Increased emphasis was placed on the study of diseases of laboratory animals, and initial work was begun on establishing acceptable "normal" values for blood chemistries, hematological values and blood groups of several laboratory animal species. The population of the animal colony increased and now totals approximately 4,000.

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Project No. 3A013001A91C

In-House Laboratory Independent

Research

Task No. 00

In-House Laboratory Independent

Research

Work Unit No. 149

Production of Polyvalent Antivenins

# Investigators (FY 1967):

#149 W. F. Kocholaty, Ph.D.
M. Edith Ledford, A.B.
T. A. Billings, B.S.
Joyce C. Goetz, B.A.
B. D. Ashley, M.S.

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#### Detail Sheet # 1

#### (U) Progress:

Several  $\gamma$ -globulins isolated from rabbits following identical immunization schedules with photochemically detoxified venoms gave varying degrees of protection in mice against the unaltered venoms (Crotalus durissus durissus, Bothrops atrox asper, Naja naja). Investigation of these discrepancies in protective action revealed that the route of administration has a decisive influence in the demonstration of the protective effect of a given antivenin. In order to arrive at a rational appraisal of the efficacy of an immune serum, both I.V. and I.P. administration should be given consideration at least as long as one is uncertain of the chemical nature, pharmacological activity and toxicity of the variety of components contained in the venom.

A study of enzymatic activities and toxicities of some thirty representatives of the families of Elapidae, Viperidae and Crotalidae is nearing completion. Electrophoretic separation of these venoms was carried out with the purpose to correlate electrophoretic patterns with enzymatic characteristics and toxicities. Immunoelectrophoretic studies of several venoms were undertaken to characterize certain venom components.

Large-scale separation of C. atrox venom resulted in an accumulation of four distinct fractions with distinct toxic, enzymatic and pharmacological characteristics.

#### Publications and/or Presentations:

Billings, T. A. A holder to facilitate the intravenous injection of mice. USAMRL Report No. 704, 1966 (DDC AD No. 645927).

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Kocholaty, W., T. A. Billings, B. D. Ashley, M. Edith Ledford and Joyce C. Goetz. Effect of the route of administration on the neutralizing potency of antivenins. USAMRL Report No. 745, 1967.

A91C 00 149 (cent)

#### Detail Sheet # 2

Kocholaty, W., Joyce C. Goetz, B. D. Ashley, T. A. Billings and M. Edith Ledford. Immunogenic response of the venoms of ferde-lance (Bothrops atrox asper) and Lacascabella (Crotalus durissus durissus) following photooxidative detoxification. USAMRL Report No. 743, 1967.

Kocholaty, W., M. Edith Ledford, T. A. Billings, Joyce C. Gretz and B. D. Ashley. Immunization studies with Naja naja venom detoxified by photooxidation. USAMRL Report No. 744, 1967.

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Kaiser, E. and H. Michl. Die Biochemie der tierischen Gifte. Franz Deutsche Wien, 1958.

Project No. 3A014501A74D

Military Psychophysiological Studies

Task No. 00

Military Psychophysiological Studies

Work Unit No. 020

Psychophysics of Visual Perception

Work Unit No. 022

Biomechanical Aspects of Performance and Performance Decrement

# Investigators (FY 1967):

#020 G. S. Harker, Ph. D.

I. Behar, Ph. D.

D. L. Kohfeld, Captain, MSC

#022 L. S. Caldwell, Ph. D.

A. J. Lloyd, Captain, MSC

G. S. Harker, Ph. D.

D. L. Kohfeld, Captain, MSC

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#### Detail Sheet # I

#### (U) Progress:

Response to the saccadic suppression paradigm by the professional community has been excellent. Several individuals have written for further clarification and indicated the intent to initiate follow-on experimentation. A review of current practice in handling amblyopia through the use of cycloplegic drugs has suggested the possibility that an optical system to reduce the interpupillary distance could facilitate the process and the development of stereoscopic vision be induced by the use of the Pulfrich pendulum and its associated filter.

The effects of variation of an electrocutaneous ready-signal upon visual reaction-time were determined. Reaction times were short when the visual signals followed electrocutaneous offset, longer where they followed onset.

The acuity of the baboon and rhesus are comparable while that of the mangabey is inferior. However, the mangabeys tested were older than the other species. To determine whether the finding is a true species difference or one of age, additional young mangabeys are being trained.

#### Publications and/or Presentations:

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A74D 00 020 (cont)

#### Detail Sheet # 2

(i

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Harker, G. S. A saccadic suppression explanation of the Pulfrich phenomenon. USAMRL Report No. 718, 1967 (DDC AD No. 648-389); presented at the Midwestern Psychological Association meeting, Chicago, Ill., 4-6 May 1967.

Harker, G. S. and O. L. O'Neal, Jr. Some observations and measurements of the Pulfrich phenomenon. USAMRL Report No. 728, 1967 (DDC AD No. 652705).

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Yarczower, M., et. al. Visual acuity in a stumptail macaque. Science, 152: 1392-1393, 1966.

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A74D 00 022 (cont)

#### Detail Sheet # 1

#### (U) Progress:

Studies of the effects of repeated static work sessions on performance have shown that the primary determinant of work decrement was the length of the work session, and that for a fixed session length the decrement per trial was relatively constant. Furthermore, recovery from the effects of work was not systematically related to the length of the rest period after the first or second period. Ratings of the effort required to maintain a work load were shown to vary systernatically with changes in the load and in the circulatory state of the muscles. In summary, variables which affected endurance were shown to have a commensurate effect upon subjective assessment of the effort required by the task. Thus, the effort scaling procedure may prove useful as a device for assessing reserve capacity well in advance of the point at which the task demands exceed the physical capabilities of the individual and performance is necessarily terminated. Data has been collected on the effect of information feedback and muscle tension on kinesthetic estimation of limb position. Preliminary analyses indicate that individuals given increased knowledge of results overcompensate and the magnitude of the position error does not decrease. There is preliminary evidence of an optimal muscle tension which results in increased kinesthetic precision.

#### Publications and/or Presentations:

Caldwell, L. S. Laboratory demonstration - dynamometry. Presented at Ergonomics Course, Occupational Health Research and Training Facility, Public Health Service, Cincinnati, Ohio, 15 May 1967.

Caldwell, L. S. and R. P. Smith. Pain and endurance of isometric muscle contractions. J. engng. Psychol. 5(1): 25-32, 1966; USA-MRL Report No. 709, 1966 (DDC AD No. 645965).

Caldwell, L. S. and R. P. Smith. Subjective estimation of effort, reserve, and ischemic pain. USAMRL Report No. 730, 1967.

Harker, G. S. Subject controlled treadmill. Presented at the Twelfth Annual Army Human Factors R and D Conference, Fort Benning, Ga., 3-4 Oct 1966.

A74D 00 022 (cont)

#### Detail Sheet # 2

Holmgren, G. L. Characteristic pace - a potential tool for vascular surgeons. USAMRL Report No. 687, 1966 (DDC AD No. 647537).

Holmgren, G. L. Speaker recognition, speech characteristics, speech evaluation and modification of speech signal—a selected bibliography. IEEE Transactions on Audio and Electroacoustics, AU-14(1): 32-39, 1966; USAMRL Report No. 692, 1966 (DDC AD No. 645074).

Holmgren, G. L. and G. S. Harker. Characteristic pace as determined by the use of a tracking treadmill. J. appl. Psychol. 51: 278-283, 1967; USAMRL Report No. 685, 1966 (DDC AD No. 645454).

Lloyd, A. J. The occurrence of synkinesis during kinesthetic positioning responses. USAMRL Report No. 720, 1967.

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Warm, J. S., R. P. Smith, and L. S. Caldwell. Effects of induced muscle tension on judgments of time. Presented (by Warm) at Southern Society for Philosophy and Psychology meeting, Roanoke, Va., 23-25 Mar 1967.

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Evans, W. O. A titration schedule on a treadmill. J. Exper. Anal. Behav. 6: 219-221, 1963.

A74D 00 022 (cont)

## Detail Sheet # 3

Muscio, B. Is a fatigue test possible? Brit. J. Psychol. 12: 31-46, 1921.

Rohmert, W. Ermittlung von Erholungspausen für statische Arbeit des Menschen. Arbeitsphysicl. 18: 123-164, 1960.

Project No. 3A014501B71P

Basic Research in Support of Military

Medicine

Task No. 01

Biochemistry

Work Unit No. 045

Biochemistry of Corticosteroid Hormones, Proteins, and Nucleic Acids

Work Unit No. 046

Mechanism of Fibrinolysis

Task No. 02

Biophysics

Work Unit No. 010

Models and Mechanisms of the Effects of Laser Radiation on Biological Sys-

tems

Work Unit No. 013

Cellular Effects of Laser Radiation

Task No. 08

Physiology

Work Unit No. 085

Psychophysiology of Vision

Work Unit No. 086

Vestibular Function and Disorientation

Work Unit No. 087

Chloroquine Retinopathy

Task No. 10

Zoology

Work Unit No. 021

The Pathology of Animal Diseases of

Military Significance

Work Unit No. 023

Immunology, Toxicology and Hema-

tochemistry of Venoms

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  M. Edith Ledford, A.B.
  T. A. Billings, B.S.
  Joyce C. Goetz, B.A.

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#### Detail Sheet # 1

### (U) Progress:

Rat liver nuclei were separated in five fractions and they interact differently with corticosterone. The acid protein and the globulin fraction show the highest binding. A very small amount of steroid is associated with the histone fraction. In the mitochondrial fractions, the uptake of cortisol or corticosterone is associated almost entirely with the particulate fractions. Three electrophoretic peaks are obtained from mitochondria and two of these peaks display combining affinity for corticosterone. The disruption of the subcellular fractions does not destroy their binding affinity for corticosteroid hormones.

#### Publications and/or Presentations:

DeVenuto, F. Interaction of progesterone and aldosterone with red blood cells of the rat. USAMRL Report No. 677, 1966 (DDC AD No. 645109); Proc. Soc. Exper. Biol. and Med. 124: 478, 1967.

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B71P 01 045 (cont)

#### Detail Sheet # 2

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Liao, S. and H. G. Williams-Ashman. An effect of testosterone on amino-acid incorporation by prostatic ribonucleoprotein particles. Proc. N.A.S. 48: 1956, 1962.

Florini, J. R. and C. B. Brewrer. Amino acid incorporation into protein by cell-free preparation from rat skeletal muscle. III. Comparison of activity of muscle and liver ribosomes. Biochemistry, 4: 253, 1965.

Pena, A., B. Dvorkin, and A. White. Acute effect of a single in vivo injection of corticol on in vitro amino acid incorporating activity of rat liver and thymic preparations. J. Biol. Chem. 241: 2144, 1965.

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	Objective - To determ					
the activatio	n of plasminogen to pla	asmin, ti	hus increasing	understanding	of dissolution	
and preventi	on of blood clots.		_			
(U) Approach - Adequately purified plasminogen and plasmin, activated by streptokinase or urokinase, will be subjected to gel filtration and N-terminal analyses to detect changes in the primary structure of the molecule. Split products, if any, will be separated and characterized by their amino acid composition.  (U) Progress (I Apr 67 - 30 Jun 67) - The technique of upward flow on Sephadex gel columns has been adapted to the preparative columns employed in obtaining plasminogen and plasmin free of low molecular weight material and suitable for studies on the activation process. The ratio of activator to ensyme precursor influences the process and varying amounts of streptokinase have been employed in activation mixtures to provide low molecular weight products for examination by amino acid analysis.						
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#### Detail Sheet # 1

### (U) Progress:

Mixtures of plasminogen and varying amounts of streptokinase were separated by gel filtration and the apparent split products examined by use of a peptide survey system. Partially separated products of activation were subjected to amino acid analysis.

Studies employing N-terminal amino acid analyses of plasminogen, plasmin and activation mixtures of the proenzyme indicated that the gel filtration process may leave a number of contaminating proteins or peptides in the preparations examined. The technique of upward flow on Sephadex G-200 columns was adapted to the preparative-size column and used in obtaining plasminogen and plasmin of suitable purity for studies on the activation process. This system also provided a separation of the low molecular weight split products produced in the activation of plasminogen by streptokinase.

A peptide survey system incorporating a Bio-Gel P-2 column was utilized to examine the separated low molecular weight constituents. The presence of substances the size of amino acids, dipeptides and tripeptides has been reasonably well established, but a constituent with a molecular weight above 2,000 remains to be characterized.

It would appear that the ratio of activator to enzyme precursor has an important influence on the process of activation and the appearance of any split products, therefore, varying amounts of streptokinase purified by gel filtration have been used in activation mixtures. The split products are being separated by column chromatography for determination of inolecular weight and amino acid composition. Preliminary studies on the amino acid content show presence of lysine and alanine with smaller amounts of arginine, histidine and glutamic acid.

#### Publications and/or Presentations:

Bobbitt, J. L. Factors affecting resolution and reproducibility of gradient elution automatic amino acid chromatograms. USAMRL Report No. 693, 1966 (DDC AD No. 645925),

B71P 01 046 (cont)

#### Detail Sheet # 2

Bobbitt, J. L. and D. E. Reed. Differentiation between proteolytic and TAME esterolytic activity in <u>Crotalus atrox</u> venom. USAMRL Report No. 712, 1966 (DDC AD No. 647148); presented (by Reed) at the Southeastern Regional Meeting of the American Chemical Society, Louisville, Ky., 27-29 Oct 1966.

Wabner, C. I., J. L. Gray and W. F. Blatt. Influence of sex and thyroid principles on the antiproteolytic activity of rat serum. Thromb. Diath. Haem. 16: 86, 1966.

#### Selected Bibliography:

Robbins, K. C., L. Summaria, B. Hsieh and R. J. Shah. The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. J. Biol. Chem. 242: 2333, 1967.

De Renzo, E. C., E. Boggiano, W. F. Barg, Jr. and F. F. Buck. Interaction of streptokinase and human plasminogen. J. Biol. Chem. 242: 2428, 1967.

Taylor, F. B., Jr. Purification and characterization of streptokinase and its interaction with plasminogen. Fed. Proc. 26: 647, 1967.

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# Detail Sheet # 1

#### (U) Progress:

During the first three quarters of FY 1967, the thermal denaturation of the intracellular microbial enzyme  $\beta$ -galactosidase by 10.6 micron laser radiation as a function of time of exposure was determined for two power densities of radiation. The experiments were designed to evaluate the relations necessary to construct a mathematical model adequate to describe threshold thermal injuries.

#### Publications and/or Presentations:

None.

#### Selected Bibliography:

Henriques, F. C. Studies of thermal injury. AMA Arch. Pathol. 43: 489-502, 1947.

Mixter, G., Jr., G. P. DeLhery, W. L. Derksen, and T. I. Monahom. The influence of time on the death of Hela cells at elevated temperature. Temperature, Its Measurement and Control in Science and Industry, New York: Reinhold Publishing Corp., Part 3, 1962, pp. 177-182.

Fugitt, C. H. A rate process theory of thermal injury. AF Special Weapons Project Report No. 606, 1955 (DDC AD No. 212660).

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Wood, T. H. Lethal effects of high and low temperatures on unicellular organisms. Adv. Biol. Med. Physics, 4: 119-165, 1956.

Davies, J. M. The effect of intense thermal radiation on animal skin. A comparison of calculated and observed burns. Quartermaster

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# Detail Sheet # 2

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Research and Engineering Command Report T-24, 1959 (DDC AD No. 456794).

Davis, T. P. A theoretical and experimental investigation of the temperature response of pig skin exposed to thermal radiation. University of Rochester Atomic Energy Project Report UR-553, 1959.

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#### Detail Sheet # 1

#### (U) Progress:

Studies of the effects on CO<sub>2</sub> output brought about by incubation of cells in various sugars or hexitols were carried out during the early part of the year. Certain of these compounds evidently affect sites also affected by increased temperatures such as laser irradiation generates. Measurements of cofactors in extracts of these cells showed no correlation between NAD levels and the incubating sugar, or CO<sub>2</sub> production. ATP content was generally below average in cells with low CO<sub>2</sub> output. Agreatly decreased decarboxylase activity occurred in some cells primarily due to enzyme loss or inhibition rather than to a thiamine pyrophosphate lack. Sugar efflux from these cells showed no differences in rates of facilitated diffusion, but cells with a high CO<sub>2</sub> output were able to bind or compartment, and thus retain, more sorbose.

#### Publications and/or Presentations:

Doyle, R. J. and E. Spoerl. Sugar retention, cofactor levels and leakage of metabolites in X-irradiated, starved yeast cells. USA-MRL Report No. 742, 1967.

Spoorl, E. and R. J. Doyle. The influence of some hexitols and sugars on CO<sub>2</sub> production by starved and X-irradiated, starved yeast cells. USAMRL Report No. 682, 1966 (DDC AD No. 645204).

Spoorl, E. and R. J. Doyle. Modification by hexitols and sugars of changes induced in yeast by starvation. I. Differences in  $CO_2$  production,  $O_2$  use and viability. USAMRL Report No. 726, 1967.

Spoerl, E. and R. J. Doyle. Modification by hexitols and sugars of changes induced in yeast by starvation. II. Cellular depletions and sugar binding. USAMRL Report No. 727, 1967 (DDC AD No. 652704).

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Green, D. E., E. Murer, H. O. Hultin, S. H. Richardson, B. Salmon, G. P. Brierly and H. Baum. Association of integrated metabolic pathways with membranes. I. Glycolytic enzymes of the red blood corpuscle and yeast. Arch. Biochem. Biophys. 112: 675, 1965.

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(U) Tech Objective - To diagnose and classify defective color vision in U.S.  Army personnel and to investigate the physiological basis of defective color vision					
Army personnel and	to investigate	the phys	iological basis	of defective c	olor vision
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(U) Approach - The Farnsworth Munsell 100 hue test and Rayleigh match equations will be used to diagnose color vision anomalies in humans. Modifications of these tests will be used with monkeys and apes to compare primate vision with human vision. The anatomical organization of the visual system will be studied with light and electron optics. The electrophysiology of the system will be studied with microand macroelectrode recordings at the level of the retina and LGN.					
(U) Progress (I Apr 67 - 30 Jun 67) - Behavioral data: Mangabey and baboon behavioral data on effect of retinal area are acceptable and articles being prepared. Chimpanzee and gibbon studies making slow progress. Physiological data: Optical system for small spot ERG and receptive field studies now in final stages of fabrication. Hope to begin data collection on small spot ERG study soon. Program for processing ERG data by computer being written.					
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#### Detail Sheet # 1

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#### (U) Progress:

Behavioral: Studies investigating the effect of retinal area (1, 2, 5, and 10°) on photopic spectral sensitivity have been completed on the mangabey and baboon. Definitive human control data for these studies are nearing completion. Similar studies on the chimpanzee and gibbon are making slow but satisfactory progress. Automation of the behavioral system to improve speed and accuracy of data collection is currently underway and fabrication of additional optical systems to investigate hue discrimination and Rayleigh match will be completed as soon as the spectral sensitivity studies are completed.

Physiological: Additional data have been added to the baboon ERG study and the manuscript is in preparation. Additional data were also collected on the mangabey lateral geniculate single unit study and final tabulations of the data are in progress. The development of necessary optics, electronics, etc., required for doing small spot ERG work is essentially complete and pilot data will soon begin. The small spot ERG investigations offer the exciting possibility of doing, for the first time, relatively precise retinal mapping with a physiological measure that could have application not only to understanding basic physiological mechanisms, but also clinically for the evaluation of various visual anomalies. Specifically, one of the purposes for developing this technique was to evaluate discrete experimentally produced laser-induced lesions.

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B71F 08 085 (cont)

#### Detail Sheet # 2

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#### Detail Sheet # 1

## (U) Progress:

Research to investigate the interaction of cerebello-vestibular structures has been initiated. Research on the effects of nembutal, amphetamine, and lidocaine upon vestibular responses is also in progress. An important question to be answered is whether habituation takes place in the absence of a nystagmic motor response. In addition, a systematic series of chronic recording and stimulating electrode implants is continuing with cats, in an attempt to delineate the neurological systems controlling habituation. To complement and hopefully verify this neurological data with anatomical evidence, histological material from cats with chronic lesions in the cerebellum is being sectioned for microscopic analysis of degeneration in the vestibular system.

Due to an almost complete lack of comparative data with reference to habituation and neurological structure, a program of comparative behavioral and physiological study has been initiated. Pilot data from kangaroo rat, and Rattus rattus indicate that rodents do not possess a "normal" nystagmic response to angular acceleration. It is hoped that further work with these animals as well as guineapigs, cats, and primates will clarify knowledge of present physiological mechanisms.

In this regard, a restraint device has been developed which will permit electrophysiological recordings from infra-human primates while both in an unanesthetized condition and undergoing acceleration. Recording from unanesthetized monkeys trained in operant techniques will make it possible to compare electrophysiological responses with subjective illusions resulting from vestibular stimulation.

A new restraint device was also developed for cat to allow chronic electrode recordings to be taken without struggle artifacts. The cat work is continuing with the prime question related to the interaction of cerebellar and vestibular structures. This has included implanting of electrodes in the reticular formation, fastigial nuclei, vestibular nuclei, and centrum medianum.

An important question to be answered is what neurological role fatigue plays in disorientation and habituation. Since it has been

B71P 08 086 (cont)

#### Detail Sheet # 2

shown that the descending and medial vestibular nuclei are responsible (necessary) for the rapid eye movement (P.EM) phase of sleep, the interaction of fatigue with vestibular responses would appear to be a most meaningful neurophysiological question. This is further supported by the fact that one of the prime physiological changes after continued slow rotation is fatigue. The operation of fixed and rotary wing aircraft and the subsequent interaction of fatigue and vestibular stimulation, may be an important problem in the field. However, little behavioral and no physiological data are presently available on this subject. Nystagmic and electrophysiological studies in both cat and man are presently underway to investigate their relationships.

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#### Detail Sheet # 1

# Publications and/or Presentations:

None.

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## Detail Sheet # 1

#### (U) Progress:

Feline panleukopenia in previous years has caused up to 50% mortality in newly arrived, random source, nonconditioned cats. This year we have carefully evaluated the efficacy of endogenous serum in protecting against the disease. Of the 81 cats included in this study, 37 received endogenous serum, 6 cats received commercially available serum and 38 received no serum. There were 14 deaths in the latter group which were diagnosed as panleukopenia on the basis of clinical signs, hematologic values and necropsy findings. Work is continuing in this area to collect more data and a publication is anticipated.

The decreased fecundity in the hairless mouse colony last year was partially due to improper husbandry procedures which have been corrected; however, it stimulated our interest in the genetics of these mice. As a result, we have adapted a squash preparation for male miotic chromosomes to our laboratory and are currently developing our techniques for tissue culture of somatic cells for evaluation of mitotic figures. Our early results would indicate some abnormality in the end to end association of the X and Y chromosomes in a number of miotic metaphase figures in hairless males. Further work is needed to substantiate or refute this observation.

We are continuing our efforts in the attempt to determine the pathogenesis of the granulomatous hepatitis noted in newly received rabbits. Current findings would indicate a small intracytoplasmic coccobacillus as the etiologic agent. This organism may be seen occasionally in hematoxylin and eosin stained sections. When visible in gram stained tissue sections, the organism assumes a gram positive to purplish appearance. It is most apparent when stained with a modified Machiavello's method.

A sporadic disease has been noted recently in young mice in our white mouse colony. Clinically one sees swollen joints which eventually progress to auto-amputation of an extremity as is present in mouse pox or infection with Streptobacillus moniliformis. Histologic preparations tend to rule out the viral etiology and attempts are being made to culture a causative agent. Pathogenic streptococci and staphylococci

B71P 10 021 (cont)

#### Detail Sheet # 2

have been isolated from internal organs and hearts blood of those animals moribund and dead.

Several new techniques and capabilities have been added to our clinical pathology section. The most important of these would be a functioning bacteriology ability. This has greatly improved our ability to monitor the health status of the animal colony.

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#### Detail Sheet # 1

#### (U) Progress:

Column chromatography on fibrous DEAE-cellulose in TRIS-HCl-buffer pH 7.2 with an altered elution gradient permitted the fractionation of the major portion of the lethal protein of C. atrox from other biologically active mater: \*1. Limited degrees of resolution of the lethal protein were found to occur using molecular sieve filtration (sephadex, sepharose, biogel) or ammonium sulfate fractionation. Inhibition of esterolytic activity with DFP did not lead to the isolation of a more toxic lethal protein, although contaminant biologic activity other than toxicity was minimized. A homogeneous lethal protein was not achieved. There is no evidence to date to suggest that the lethal protein corresponds to any degradative enzyme to be present in C. atrox venom. A USAMRL report, "Further observations on the properties of the lethal protein in Crotalus atrox venom," has been written and is presently undergoing minor revisions.

#### Publications and/or Presentations:

Ashley, B. D. and P. M. Burchfield. Maintaining a snake colony for venom collection. USAMRL Report No. 696, 1966 (DDC AD No. 647149).

Ashley, B. D. and P. M. Burchfield. Biology of snakes. Lecture given to the Meeting of the 4th District Education Association Science Teachers, Elizabethtown, Ky., 14 Oct 1966.

Ashley, B. D. and R. M. Burchfield. Current recommendations for first aid and inedical care of victims of snake envenomation and prevention of biles. Lecture given to the Hardin-LaRue County Medical Society Meeting, Elizabet town, Ky., 5 Jan 1967.

Ashley, B. D. and D. P. Thompson. Snakes and snakebite. Lecture given to the Pre-Medical Fraternity Meeting, University of Louisville, Louisville, Ky., 20 Mar 1967.

Ashley, B. D., P. M. Burchfield and D. P. Thompson. Venomous snakes and snakebites in Kentucky. Lecture given to the Ky. State Association of Medical Assistants, Park City, Ky., 13 May 1967.

B71P 10 023 (cont)

#### Detail Sheet # 2

Bobbitt, J. L. and D. E. Reed. Differentiation between proteolytic and TAME esterolytic activity in <u>Crotalus atrox</u> venom. USAMRL Report No. 712, 1966 (DDC AD No. 647!48); presented (by Reed) at Southeastern Regional Meeting of the American Chemical Society, Louisville, Ky., 27-29 Oct 1966.

Burchfield, P. M. Instruction of personnel departing for Vietnam. Lecture given to the POR/POM Course, 4th Battalion (Mechanized), 54th Infantry, Fort Knox, Ky., Nov 1966.

Reed, D. E. The resolution of enzymatic activities in Crotalus atrox venom by chromatography on DEAE-cellulose. USAMRL Report No. 705, 1966 (DDC AD No. 645904).

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Pfleiderer, G. and G. Sumyk. Separation of rattlesnake venom proteinases by cellulose ion-exchange chromatography. Biochim. Biophys. Acta, <u>51</u>: 482, 1961.

Peterson, E. A. and H. A. Sober. Variable gradient device for chromatography. Anal. Chem. 31: 857, 1959.

Reed, L. J. and H. Muench. A simple method of estimating fifty per cent end points. Amer. J. Hyg. 21: 493, 1938.

Project No. 3A014501B71R	Research in Biomedical Sciences
Task No. 01	Surgery
Work Unit No. 100	Acute and Chronic Effects of Laser Radiation on Mammalian Tissues
Work Unit No. 101	Ocular Effects of Laser Radiation
Work Unit No. 102	Effects of Laser Radiation on Im- mune Mechanisms
Work Unit No. 103	Cutaneous Burns Induced by Laser Radiation
Work Unit No. 104	Effects of Laser Radiation on the Mammalian Hematopoietic System
Work Unit No. 105	Effects of Laser Radiation on Active Transport in Living Membranes
Work Unit No. 275	Laser Effects on Performance
Work Unit No. 276	Instrumentation Design

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R. W. Bull, Captain, VC
R. S. Fisher, Captain, MC
D. K. Hysell, Captain, VC
G. R. Peacock, Captain, MSC
H. M. Leibowitz, Captain, MC
A. H. Bryan, Captain, MC
G. H. Herbener, M.S.
A. J. Luzzio, Ph.D.

#103

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W. H. Parr, Ph.D.

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R. S. Fisher, Captain, MC

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S. H. Revusky, Ph.D.

J. F. Medley

#276

J. C. Rosenbaum, Jr., M.S.

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B. REVNORUS Laser, Animal Studies; Las Thermal; Retina; Eye; Eye Burns;	er İnjury Eye İnjur	Biologic	al; A	cute; Chron	iic; Radia	tion,

- (U) Tech Objective To appraise effects of laser irradiation on various Usaues and organs and biological materials with emphasis on acute and long-term effects.
- (U) Approach Changes occurring in rat corneal epithelial cells due to ruby and CO<sub>2</sub> laser irradiation will be investigated and correlated with absorbed dose. Effects of high doses of ruby, Nd, and CO<sub>2</sub> laser radiation on animal eyes will be studied and related to whole body effects. Studies of modes of interaction of laser radiation with bio-materials will be conducted.
- (U) Progress (1 Apr 67 30 Jun 67) Previous experiments in which aberrant cells were produced in the cornea of rats by ruby laser radiation have been repeated. Other preliminary studies indicate that the number of aberrant cells produced using a constant power density will depend on the pulse length of the beam. A laboratory report of kidney damage as a consequence of laser irradiation and the subsequent effects in the body as a whole is being prepared. A model, based on surface temperature increases, has been formulated and compared with experimental laser injury data reported in the literature and gathered in this laboratory.

Peacock, G. R. Surface temperature as a parameter in estimating laser injury thresholds. USAMRL Rep. No. 733, 1967.

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## Detail Sheet # 1

## (U) Progress:

Laser irradiation of renal and ocular structures in animals revealed the following: a) Biweekly lasing (6943 A) at 12.5 j/cm² and 6.0 j/cm² of translocated kidneys in rats resulted in gross damage to overlying skin and kidneys at the higher energy level and in microscopic damage at the lower level. A decrease in urinary output with proteinuria and loss of body weight was also noted. b) Studies using a small number of animals indicate that atypical cells may still persist in the corneal epithelium of the rat 180 days after exposure to ruby laser radiation (4 j/cm²). On the basis of comparison with CO<sub>2</sub> laser effects, the production of atypical cells are thought to be a thermal effect. Preliminary threshold values obtained for the production of these aberrant cells are between 0.5 and 1.0 j/cm² for a 3 msec pulse of ruby laser radiation and approximately the same for a 40 msec pulse of CO<sub>2</sub> laser radiation.

Gress in vivo evaluation of rat eyes immediately after exposure to 4 j, 8 j and 12 j/cm<sup>2</sup>, showed hemorrhages and bubbles in the anterior chamber and a steamy opacity of the cornea which appeared to be dose dependent. After 30 days, nearly complete recovery from ocular inflammation had occurred at the lower dose level while at the higher levels massive hemorrhages were seen in the anterior chamber. In pigmented rabbit eyes, energy densities as low as 0.1 j/cm<sup>2</sup> (normal pulse, collimated, 6943 Å) incident on the cornea caused retinal destruction and vitreous hemorrhage.

#### Publications and/or Presentations:

Bull, R. W. Ruby laser's biological effect on the kidney and skin of hooded Long-Evans rats. Presented at the Gordon Research Conference, Lasers in Medicine and Biology, Andover, N. H., 19-23 Jun 1967.

Fine, S., E. Klein, W. H. Parr, B. Fine, R. S. Fisher and G.R. Peacock. Hazard studies with lacer radiation. Presented (by S. Fine) at the Third DOD Laser Conference on Laser Technology, Pensacola, Fla., 18 Apr 1967.

B71R 01 100 (cont)

## Detail Sheet # 2

Fisher, R. S. and W. H. Parr. The fundamental concepts of laser radiation and the bi-logical effects of laser radiation. Presented (by Fisher) at the Health Physics Society - Midwest Chapter's Joint Meeting, Cumberland Falls, Ky., 9-11 Sep 1966.

Jones, A. E. and A. J. McCartney. Ruby laser effects on the monkey eye. Invest. Ophth. 5(5): 474-483, 1966.

Leibowitz, H. M. Medical aspects of incapacitation. Presented at the US Army Conference on Laser Eye Protection, Natick, Mass., 24 May 1967.

Parr, W. H. and R. S. Fisher. Aberrant corneal epithelial cells produced by ruby laser irradiation. USAMRL Report No. 698, 1966 (DDC AD No. 645452); presented (by Fisher) at the Northeast Electronics Kesearch and Engineering Meeting, Boston, Mass., 2-4 Nov 1966.

Parr, W. H. and G. R. Feacock. Corneal damage from laser radiation. Presented (by Parr) at the Gordon Research Conference, Lasers in Medicine and Biology, Andover, N. H., 19-23 Jun 1967.

Parr, W. H., G. R. Peacock and R. S. Fisher. Laser effects on the corneal epithelium. Presented (by Parr) at the IEEE Ninth Annua. Symposium on Electron, Ion, and Laser Beam Technology, Eerkeley, Calif., 9-11 May 1967; presented (by Parr) at the US Atomic Energy Test Site, Mercury, Nev., 12 May 1967.

Peacock, G. R. Laser hazards and safety. Presented at the Junior Science and Humanities Symposium, Fort Knox, Ky. 1 Apr 1967.

Peacock, G. R. Medical aspects of incapacitation. Presented at the US Army Conference on Laser Eye Protection, Natick, Mass., 24 May 1967.

Peacock, G. R. Surface temperature model for laser injury. Presented at the Gordon Research Conference, Lasers in Medicine and Biology, Andover, N. H., 19-23 Jun 1967.

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# Detail Sheet # 3

Peacock, G. R. Surface temperature as a parameter in estimating laser injury thresholds. USAMRL Report No. 733, 1967.

# Selected Bibliography:

Proceedings of the First Annual Conference on Biological Effects of Laser Radiation. Fed. Proc. 24: Suppl. 14(2), Jan-Feb 1965.

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Ransom, R. G. The influence of ionizing radiation on mitosis in the corneal epithelium of the rat. USAMRL Report No. 110, 1953 (DDC AD No. 20875).

Gay, H. and B. P. Kaufman. The corneal epithelium as a source of mammalian somatic mitoses. Stain Tech. 25: 209, 1950.

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Lacr, Y., C. Simpson, E. Klein and S. Fine. The pathology of lase: irradiation of the skin and body wall of the mouse. Amer. J. Path. 47(4), Oct 1965.

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## Detail Sheet # 1

#### Publications and/or Presentations:

None.

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# Selected Bibliography:

Jones, A. E. and A. J. McCartney. Ruby laser effects on the monkey eye. Invest. Ophth. 5. 474, 1966.

Ham, W. T., H. Weisinger, F. H. Schmidt, R. C. Williams, R. S. Ruffin, M. C. Schaffer and D. Guerry, III. Flash burns in the rabbit retina. Amer. J. Ophth. 46: 700, 1958.

Jacobson, J. H., Blossom Cooper and H. W. Najac. Effects of thermal energy on retinal function. Technical Documentary Report No. AMRL-TDR-62-96-1962.

Foulds, W. E. Experimental retinal detachment. Tr. Ophth. Soc. U. Kingdom, 83: 153, 1963.

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Total serum protein was not affected. The animals have been sacrificed and microscopic sections of spleens will be examined for histological study. The above experiments are currently being repeated for confirmation of the data.  **Committee Note   1	sponses are e	nhanced	when the antig	er is inj	ected one	hour after le	ser treatme	ent. No
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# Detail Sheet # 1

# (U) Progress:

Measurements on a number of rabbits showed that the spleen of a rabbit could be located accurately with the xiphoid process as a reference point. Several groups of rabbits were exposed to various levels of ruby laser radiation through the intact skin at the splenic site determined by the method briefly described above. One hour after exposure, each animal was injected subcutaneously with bovine serum albumin (BSA). Unlased rabbits were also injected and served as a control group. All animals were bled at periodic intervals before and after exposure and serum anti-BSA levels were determined by the quantitative precipitin method. Forty days after initial exposure and injection, a second laser exposure was given each animal at the same site followed by a second BSA injection one hour later. Anti-BSA concentrations were determined for each animal at periodic intervals following radiation.

Thus far, the results show that no significant differences occurred in the primary and secondary immune responses of rabbits exposed to 100-200 joules of ruby laser radiation. Anti-BSA appeared to increase in the primary immune response after 400 joules of radiation and a greater increase in anti-BSA occurred during the secondary response after an additional 400 joules, or a total of 800 joules, had been delivered.

The laser injury to the rabbits was restricted to a circular area 1 cm in diameter and was marked by a slight erythema with no swelling of the skin. At autopsy, 60 days after exposure, gross and microscopic pathology showed no evidence of injury to the spleen or surrounding organs, nor any suggestions of repair.

At this time, due to wide variations in immune responses between individual animals, a statistical analysis of the data does not support the final conclusion that laser radiation of the spleen enhances the immune response. However, since trend effects are apparent, these experiments are being repeated, with some modifications, to reduce bic logical variations so that a valid statistical analysis can be made. B71R 01 102 (cont)

# Detail Sheet # 2

Publications and/or Presentations:

None.

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Selected Bibliography:

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#### Detail Sheet # 1

# (U) Progress:

The dose-response relationship for cutaneous burns, ranging from minimally detectable erythema to partial tissue coagulation, has been determined for a limited range of CO2 laser radiation power densities and exposure times. The data to date indicate that the dose-response relationship for all measured degrees of injury within this power density range (1 to 8 watts) may reasonably be described by simple power functions. However, limited data suggest that these relationships may change outside this power density range. The best mathematical fit of the data will be determined after experiments at high and lower power densities. Numerous biopsies are now being processed and analyzed by histochemical methods to evaluate depth of tissue change as a function of power density and exposure time.

# Publications and/or Presentations:

Brownell, A. S., W. H. Parr, D. K. Hysell, and R. S. Dedrick. Threshold lesions induced in porcine skin by CO<sub>2</sub> laser radiation. USA-MRL Report No. 732, 1967; presented (by Brownell) at the Gordon Conference on Lasers in Medicine and Biology, Andover, N. H., 19 Jun 1967.

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#### Detail Sheet # 1

#### (U) Progress:

1) Investigations appraising the side effects associated with lased blood in the circulatory system indicate that a large percentage of in vitro lased (13 j/cm²) red cells reinjected into the donor dog are cleared from the peripheral circulation within the first two hours or less. Preliminary studies showed little or no effect on whole blood lased with energy densities from 5 - 10 j/cm². 2) Isolated rat bones were fractured with energy densities of 190 j/cm², and bone marrow extruded from the ends of the bones (no fractures evident) at approximately one-half the preceding dose. Discoloration of the bone occurred at 40 j/cm². Transducers were acquired and calibrated to measure intramedullary pressure produced by the incident laser energy.

## Publications and/or Presentations:

None.

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Cooper, M. and C. A. Owens. Labeling human erythrocytes with radiochronium. J. Lab. Clin. Med. 47: 67, 1956.

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23. KEYRONOS Laser; Membranes; Active Transport

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(U) Tech Objective - Effect of laser exposure on ion transport in living membranes.

(U) Approach - Isolated frog corneas and segments of frog skin are mounted in Usering than the charge and short circuit current

NA

sing type chambers and the electrical potential difference and short circuit current measured. The tissues are then lased with a ruby laser (6943 Å).

(U) Progress (1 Jul 66 - 12 Aug 66) - 1) Cornea. Immediately post-lasing there are large increases in the short circuit current (which is equivalent to the active chloride transport) and a transient decrease in the potential difference. In the steady state 1 hour post-lasing the short circuit current was still an average of 18% above control levels. These increases in current appear to be cumulative with repeated lasing of the same cornea. 2) Frog skin. There is a net increase in the short circuit current (which is equivalent to the active sodium transport) following lasing of the endotherial side of the skin with little effect on the potential difference. Lasing of the epithelial side produces a 60% average decrease in potential differences with no effect on the short circuit current.

These changes are of a different nature from those of simply heating the tissue and bathing fluids which give a linear decrease in short circuit current and potential difference. Recovery from heating is to about 60% or control levels.

It appears that ruby laser radiation is producing a sustained increase in the active transport of chloride lons in the cornea and sodium and potassium ions in the frog skin. These findings are consistent with reversible and irreversible alteration in the permeability of the membranes involved.

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## Detail Sheet # 1

# Publications and/or Presentations:

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Selected Bibliography:

Photophysiology, edited by A. C. Giese, Vols. I and II. New York: Academic Press, 1964.

Rehm, W. S., T. L. Davis, C. Chandler, E. Gohmann, Jr. and A. Bashirelahi. Frog gastric mucosae bathed in chloride-free solutions. Amer. J. Physiol. 204: 233-242, 1963.

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the anesthesia requir	ed to subdue t	the anim	al prior to lase	er exposure.	Thus, we will	
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## Detail Sheet # 1

#### (U) Progress:

If an animal becomes sick after it eats something, it will develop an aversion to whatever it ate. In the case of X-irradiation, such food aversions are the single most sensitive behavioral technique for the detection of symptomatology in animals since such aversions occur if irradiation at 10% of the LD-50 follows food consumption. Thus, it seemed possible that a similar technique might permit detection of laser injury. Pilot work on this possibility has provided basic experimental design data. Edema, a frequent consequence of laser mjury, was produced in rats by intraperitoneal injection of isotonic glucose after they had consumed saccharin solution. This alone did not produce aversion. Sodium pentobarbital injection into rats after they had consumed saccharin solution also did not produce aversion. Thus, anesthesia can probably be used in conjunction with detection of laser injury by means of food aversions. (The failure of some potent physiological assaults to produce food aversions led us to make sure we could obtain X-ray produced aversion under the setting factors in our laboratory; we could obtain them.) Thus, this technique used with scheduled laser irradiations conceivably will detect specific behavioral responses to laser irradiation as well as general stress disturbances.

# Publications and/or Presentations:

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Revusky, S. H. Aversions to saccharin produced by post-ingestive nausea; implications to drive theory. Presented at the Psychonomic Society meeting, St. Louis, Mo., 29 Oct 1966.

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Revusky, S. H. Hunger level during food consumption: effects on subsequent preference. Psychon. Sci. 7: 109-110, 1967; USAMRL Report No. 681, 1966 (DDC AD No. 645207).

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B71R 01 275 (cont)

#### Detail Sheet # 3

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Revusky, S. H. Some statistical treatments compatible with individual organism methodology. J. exper. Anal. Behav. 10: 319-330, 1967.

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m. (U) Approach - 1) To develop techniques for the application of transducers and as-							
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pressure changes in biological systems resulting from absorbed laser energy. 2) To							
develop a device for the absoluse m	easuren	nent and contin	uous monitori	ng of laser en-			
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system efficiency.							
1 · · · · · · · · · · · · · · · · · · ·							
(U) Progress (1 Apr 67 - 30 Jun 67) - 1) A constant flow calorimeter using a glass							
body and a silver chloride window has been developed to monitor CW laser output at							
*10.6 µ. Additional calorimeters are in various stages of design and development with							
absolute calibration pending delivery of ordered standards. 2) Mechanisms to control							
and measure pulsed outputs, approximately . 1 - 60 sec in length, to .01 sec, have							
been installed in the CO <sub>2</sub> laser. 3) Modifications to include neodymium and CO <sub>2</sub> "Q"							
switching are awaiting delivery of ordered components. 4) Pulse forming networks							
have been installed providing a selection of 12 flash lamp pulse lengths varying from							
approximately 1 millisecond to 5 milliseconds.							
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#### Detail Sheet # 1

## (U) Progress:

1) Calorimeters of various design, with and without constant flow capabilities, incorporating such features as modified metallic cones, selective window material and absorbing fluids for specific spectral response have been constructed. Additional calorimeters are in various stages of design and development with absolute calibration pending delivery of ordered standards. 2) Mechanisms to control and measure pulsed outputs, approximately 0.1 - 60 sec in length have been installed in the CO<sub>2</sub> laser. 3) Modifications providing a "Q" switch mode for the ruby laser have been completed. "Q" switch capabilities at neodymium and CO<sub>2</sub> wavelengths are awaiting delivery of ordered components. 4) Pulse forming networks have been installed providing a selection of 12 pulse lengths varying, for ruby, from approximately 0.4 to 4.0 msec with a peak power of approximately 85 kw.

# Publications and/or Presentations:

None.

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Project No. 3A014501B74C

Task No. 00

Basic Research in Performance Effectiveness

Basic Research in Performance Ef-

fectiveness

Work Unit No. 030

Audition and Auditory Perception

# Investigators (FY 1967):

#030

M. Loeb, Ph.D.

J. L. Fletcher, Lt Colonel, MSC

W. J. Gunn, B.S.

J. N. Cronholm, M.S.

J. L. Hatfield, Major, MSC

D. L. Kohfeld, Captain, MSC

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(U) Tech Objective - To	determine	the me	chaniems by w	hich the huma	n observer					
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(U) Approach - Research	ch is in two	areas;	.从), middle car	function; 2) as	iditory per-					
ception, e.g., vigilance,	the utilizat	ion of in	itensity and ter	mporal cues, t	he rale of					
prior experience, auditory	y localizati	on, and	masking.							
m. (U) Progress (1 Apr 67	~ 30 Jun 6	7) - Exp	eriments en c	onditioning and	inhibition of					
the acoustic reflex have ge	enerally be	en negat	iv . This is a	ome evidence	that attention					
to a contra-aural stimulus	changes th	he magn	it do of the ac-	oustic reflex.	Apparatus					
for localization study has l	been compl	leted and	laf st stage	of evaluation i	s complete.					
Experiments on effects of	signal and	stimulu	s rate on vigil:	ance suggest th	at the latter					
is more important. Subjec	ts respond	ing to a	Experiments on effects of signal and stimulus race on vigilance suggest that the latter							
is more important. Subjects responding to several signal levels perform similarly to those responding to one. An experiment on changing signal rate is completed, and										
those responding to one.	An expecim	ing to se	everal signal le Changing signal	trate is compl	similarly to					
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#### Detail Sheet # 1

## (U) Progress:

Recent experiments on auditory vigilance suggest that performance while attending to signals of several intensities closely resembles that while attending to a single level. Experiments on changes of signal rate within a session are continuing. Preliminary studies of the effect on acoustic reflex (AR) function of attending to a faint auditory stimulus indicate that AR functioning is enhanced. In a study on relationships for detection of auditory and visual stimuli, it was concluded that there are correlations of indices of criteria for responding and of sensitivity when the tasks were made sufficiently similar. An experiment on the role of the pinna in auditory localization is planned for the near future.

## Publications and/or Presentations:

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#### Detail Sheet # 2

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von Békésy, Georg. Experiments in Hearing. New York: McGraw-Hill, 1960.

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Project No. 3A025601A819

Army Aviation Medicine

Task No. 00

Army Aviation Medicine

Work Unit No. 015

The Measurement, Composition, and Stability of Complex Skills

Work Unit No. 016

Disorientation and Performance

Work Unit No. 017

Traumatic Origins of Hearing Loss

# Investigators (FY 1967):

#015 M. J. Herbert, Ph.D. M. R. Harris, Captain, MSC J. L. Hatfield, Major, MSC

#016 J. H. Brown, Ph. D.
J. E. Marshall, Captain, MSC

#017 J. L. Fletcher, Lt Colonel, MSC M. Loeb, Ph. D. I. Behar, Ph. D. W. J. Gunn, B.S.

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## Detail Sheet # 1

#### (U) Progress:

Performance by 107 subjects on 35 lab-based apparatus and printed tests of a number of skill aptitudes has been correlated and factor-analyzed. From the analysis, two papers are in preparation. One reports a new and more effective measure of response orientation; the other reports on a new, simple test of aptitudes best measured by the SAM two-hand coordination apparatus in the past. The second phase of a hand-steadiness study was aborted because of a failure in recording apparatus. This completion is programmed for FY 1968. A test of kinesthetic sensitivity was evaluated on an N of 60. Test reliability was considered too low, and led to a change in test procedure from simultaneous to successive judgment of presented weights. An N of 61 subjects has been reached at reporting date. A subject N of 73 has been reached in the vehicle-skill study based on performance on the lab-based reference measures and the revised driving performance battery. Ninety-one transportation drivers have been tested on the driving battery in the study of traffic violation. A limited version of the driving performance battery was constructed at the US Primary Helicopter Center at 'ort Wolters, Texas. One-hundred three student pilots showing: tisfactory flying proficiency, and 32 "failures" or "set-backs" have completed performance on the battery and on ten lab-based skill reference tests.

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#### Detail Sheet # 2

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hot only from	very intense stimula	tion of the	vestibular sy	stem, but a¹so	from very				
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have been ini	tiated. In addition, n	ormal sub	jects are pres	ently being les	ted to deter-				
	icant threshold shifts								
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Aerospace Me	edicine, Johnsville, F	Pa., 7-9 F	Feb 67; Marsha	ill, J. E. Ger	eralization of				
nystagmic ha	bituation as a function	of stimu	lus intensity.	Presented at t	he 38th An-				
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#### Detail Sheet # 1

#### (U) Progress:

The assessment of subjective velocity via psychophysical techniques is continuing and a study utilizing auditory cross-modality matching was completed and has been submitted for publication. The habituation of vestibular responses to interacting stimuli was examined and found to provide a reliable means of assessing asymmetry. Two experiments determining the degree to which habituation will generalize to higher and lower intensities of angular acceleration were completed. Experiments specifying the nature and extent of adaptation in both nystagmic and subjective responses to angular acceleration have also been completed. Several baseline studies utilizing aviation personnel assigned to Fort Knox have been initiated. In addition, subjects are presently being tested to determine if threshold shifts occur with habituation.

Psychophysical scaling of subjective velocity (auditory cross-modality estimates of angular velocity during constant angular acceleration) validated the exponent of 1.0 found previously with numerical magnitude estimates. Comparable adaptation was found and it was concluded that this adaptation is a real phenomenon and of potential significance as one form of disorientation.

The extent to which habituation will transfer or generalize to higher and lower stimulus intensities and durations was determined. As with other parameters of habituation, a high degree of specificity of habituation was found. That is, there appears to be only limited generalization of habituation to stimuli that are either more or less intense or longer or shorter in duration than the habituating stimulus. However, there was an asymmetrical generalization gradient such that relatively more transfer was found from higher intensity and longer duration stimuli to accelerations of lower intensity and shorter duration than vice versa.

Arrangements have been made with the Aviation Group presently assigned to Fort Knox for volunteers to serve as subjects in several baseline studies comparing the vestibular sensitivity of Army rotary and fixed wing pilots with the sensitivity of both normal subjects and pilots of high-performance aircraft. Both nystagmic and a variety of subjective responses, including threshold judgments and magnitude

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## Detail Sheet # 2

estimates, are being recorded under several experimental conditions. These data will be related to total hours flight experience, present flying activity, and a number of other measures to be taken from the Army Disorientation Questionnaire currently being sent to all aviation units by USABAR.

In a study recently completed and currently being analyzed, subjects were tested to determine if threshold shifts are evident after extensive exposure to an accelerative environment.

Modifications of the large turntable are in progress in order to experimentally approach the question of whether "fatigue" increases one's susceptibility to disorientation. A classical view of fatigue is that it represents a regression from a state in which the individual functions primarily with reflex-like motor skills to a condition where he is more dependent upon his perceptual environment. Since disorientation is primarily a perceptual phenomenon, it is anticipated that a fatigued individual will be more likely to become disoriented.

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### Detail Sheet # 1

### (U) Progress:

Data are being collected on TTS from impulse exposure with SPL held constant while varying pulse duration. Field evaluation of the CHAPARRAL missile system is underway at White Sands Missile Test Center. Effect of meningitis on high frequency hearing was studied and reported. Work continues on monkey and human TTS from octave band exposure. Investigation is being started of high frequency effects of ototoxic drug therapy in humans, joint research with University of Colorado Medical Center. Some experiments on central effects on TTS are being completed and experiments on recovery from high frequency hearing loss are underway.

### Publications and/or Presentations:

Fletcher, J. L. and A. B. Cairns. Recovery from impulse noise induced acoustic trauma. J. aud. Res. 7: 35-39, 1967; USAMRL Report No. 686, 1966 (DDC AD No. 645898).

Fletcher, J. L. and M. Loeb. An exploratory study on the effect of pulse duration on temporary threshold shift produced by impulse moise. USAMRL Report No. 680, 1967 (DDC AD No. 647540); presented (by Fletcher) at Acoustical Society of America meeting, Los Angeles, Calif., 2-5 Nov 1966.

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Loeb, M., J. L. Fletcher, and C. E. Guthrie. A preliminary evaluation of the hazard to hearing produced by the CHAPARRAL missile, Feb 1967.

Loeb, M. and I. Behar. Temporary threshold shifts in rhesus monkeys produced by exposure to octave bands of noise. Presented

A819 00 017 (cont)

#### Detail Sheet # 2

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(by Loeb) at Acoustical Society of America meeting, Washington, D.C., 19-22 Apr 1967.

Smith, R. P. and M. Loeb. Several experiments on central factors in auditory fatigue. Presented (by Smith) at Acoustical Society of America meeting, New York, N. Y., 5 Apr 1967.

Zislis, T. and J. L. Fletcher. Relation of high frequency thresholds to age and sex. J. aud. Res. 6: 189-198, 1966; USAMRL Report No. 702, 1966 (DDC AD No. 645082).

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Kryter, K. D. and G. R. Garinther. Auditory effects of acoustic impulses from firearms. Acta Oto-laryngol. Suppl. 211, 1966.

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Ward, W. D. Effect of temporal spacing on temporary threshold shift from impulses. J. acoust. Soc. Amer. 34: 1230-1232, 1962.

Project No. 3A025601A821	Combat Surgery
Task No. 00	Combat Surgery
Work Unit No. 155	Study of Bank Blood Preserved in Acid-Citrate-Dextrose and Acid-Citrate-Dextrose with Adenine After Forty-Two Days of Storage
Work Unit No. 156	Study of Group O Blood Donors in the Military to Delineate Titers and Technic of Natural and Immune Anti- bodies Applicable to a Safe, Wide- scale Employment of O Blood to A, B, AB and O Recipients
Work Unit No. 157	Study of Transport and Logistic Prob- lems of Stored Whole Blood and Blood Components in the Military
Work Unit No. 158	Evaluation of Automated, Semiauto- mated, and Other Techniques Involved in Military Blood Banking
Work Unit No. 159	Evaluation of Mass Blood Grouping in the Military Services by Automated and Semiautomated Methods
Work Unit No. 160	Blood Groups, Blood Group Substances and Chromosome Studies of Laboratory Animals
Work Unit No. 161	Biochemical Alterations of Human Red Blood Cells in Cold Storage
Work Unit No. 162	Alteration in Protein Components of Stored Red Blood Cells
Work Unit No. 163	Pasteurella pestis and Human Blood Cross-Reacting Antigens
Work Unit No. 164	Evaluation of Methods and Models for Measuring Transfusion Effects and Practices

## Investigators (FY 1967):

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- #155 C. E. Shields, Lt Colonel, MC
  F. R. Camp, Jr., Lt Colonel, MSC
  L. J. Reed, Captain, MC
  L. G. Dauber, Captain, MC
  H. F. Bunn, Captain, MC
  R. W. Bull, Captain, VC
  S. D. Litwin, Captain, MC
- #156 F. R. Camp, Jr., Lt Colonel, MSC C. E. Shields, Lt Colonel, MC L. J. Reed, Captain, MC L. G. Dauber, Captain, MC S. D. Litwin, Captain, MC
- #157 C. E. Shields, Lt Colonel, MC
  F. R. Camp, Jr., Lt Colonel, MSC
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- #158 F. R. Camp, Jr., Lt Colonel, MSC C. E. Shields, MC
- #159 F. R. Camp, Jr., Lt Colonel, MSC C. E. Shields, Lt Colonel, MC
- #160 R. W. Bull, Captain, VC R. S. Dedrick, Captain, VC H. F. Bunn, Captain, MC
- #161 F. DeVenuto, Ph.D.
- #162 W. F. Kocholaty, Ph.D. J. L. Gray, B.S. M. Edith Ledford, A.B.
- #163 A. J. Luzzio, Ph. D.
- #164 H. F. Bunn, Captain, MC S. D. Litwin, Captain, MC C. E. Shields, Lt Colonel, MC

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#### Detail Sheet # 1

## (U) Progress:

A study comparing ACD and CPD anticoagulant with and without adenine following storage for 28, 35 or 42 days has been carried out. Results of the investigation were presented at the American Association of Blood Banks meeting in October 1966 and have been prepared for publication. This study is also described in USAMRL Report No. 719, dated 21 March 1967. A second program has consisted of giving autologous and homologous transfusions under varied conditions representing type specific and/or universal donor transfusions. A total of 185 recipients are in the process of being studied and reports are being prepared. A program to investigate blood transfusions with and without adenine in Vietnam was initiated and scheduled to be carried out in April; however, this was postponed. Supplementary and ancillary studies are being planned in preparation for patient studies in various hospitals in the country.

## Publications and/or Presentations:

Bunn, Howard F. A proposed mechanism for the glomerular filtration of hemoglobin. Presented at the American Society of Clinical Investigation meeting, Atlantic City, N. J., 30 Apr - 3 May 1967.

Bunn, Howard F. The effect of sulfhydryl reagents on the binding of human hemoglobin to haptoglobin. USAMRL Report No. 735, 1967.

Camp, Frank R., Jr. and Charles E. Shields. Comparison of blood preservation after storage in ACD or CPD solutions supplemented with adenine. Presented (by Shields) at American Association of Blood Banks meeting, Los Angeles, Calif., 25-28 Oct 1966.

Camp, Frank R., Jr. Served as instructor at course entitled "Advances in Medical Laboratory Procedures;" presented talk on "Blood Bank Operations," Walter Reed Army Institute of Research, 15 Nov 1966.

Camp, Frank R., Jr. Comparison of blood preservation after storage in ACD or CPD solutions supplemented with adenine. Presented

#### Detail Sheet # 2

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tified they will be monitored to eliminate the contaminating substance.  (U) Approach - 1st Stage: Blood will be collected from a representative military population and the presence of and titers of natural and immune anti-A and anti-B hemolysins in this blood will be assayed. Calculations of antibody titers will be based on standard blood group serology procedures. 2d Stage: After being assayed, these bloods will be transfused into A, B, and AB recipients and monitored for adverse reactions. The titers causing no reactions will be declared safe.  (U) Progress (1 Apr 67 - 30 Jun 67) - Identification of several levels of group O interference has been made in various portions of the military population. This has been clearly related to the plague immunizations and has been reported at the AMA and the Int. Soc. of Blood Transfusion. During the course of investigation of other vaccines, hemolysins to group A were also found in influenza. This is planned for presentation at the AABB, Oct 67, with the following conclusion: Hemolysin inhibition assays of influenza and plague vaccines revealed Asubstance concentrations of 12 µg/ml and 68µg/ml, respectively. These results suggest that: 1) minute amounts of A substance stimulate ABO antibody production, 2) since influenza vaccine is freely and frequently prescribed, resultant antibody production may contribute to the "naturally-occurring" ABO antibodies, 3) hemolysins may be the most sensitive of the ABO antibodies to immune stimulation, 4) administration of these vaccines to potential universal donors, young females and particularly pregnant women may be undesirable. The identification of the primary substance as plague vaccine has allowed rescheduling of the immunization program, providing a greater pool of useful group O donors of the Army Blood Program. Preliminary studies of transfusion of group O blood into non-group O recipient is in progress with subsequent work extending these studies using blood with know, antibody levels.								
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## (U) Progress:

Identification of several levels of group O interference has been made in various portions of the military population. This has been clearly related to the plague immunizations and has been reported at the American Medical Association meeting and the International Society of Blood Transfusion. The identification of the primary substance as plague vaccine has allowed rescheduling of the immunization program providing a greater pool of useful group O donors of the Army Blood Program. Transfusion of group O blood into non-group O recipients has been carried out in the first phase and is still under analysis.

## Publications and/or Presentations:

Camp, Frank R., Jr. and Charles E. Shields. Military blood banking - identification of the group O Universal Donor for transfusion of A, B and AB recipients - an enigma of two decades. Presented (by Camp) at the 11th Congress of the International Society of Blood Transfusion, Sydney, Australia, 24-29 Aug 1966; USAMRL Report No. 678, 1966 (DDC No. 645447); Mil. Med. 132(6): 426-429, 1967.

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# (U) Progress:

Equipment: Special cards containing specific dried blood group identification reagents produced by an American manufacturer were tested. Tests of veracity, avidity and reliability of reagents and packaging were made under simulated combat conditions, such as high heat-high humidity; high heat-low humidity; and various cold environments as well as following prolonged storage in high heat or total water immersion. The data indicated that the use of the equipment was not simple and was subject to environmental effects as well as being inefficient in terms of technician time. The major blood group reagents were satisfactory, but the Rh factor identification was unsatisfactory.

These cards were compared to those obtained from an European manufacturer. A report on this has been prepared and accepted for publication in Military Medicine.

# Publications and/or Presentations:

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## Detail Sheet # 1

## (U) Progress:

A system for control, distribution and prediction of requirements for blood logistics has been investigated. An applicable system of data retrieval using small computers and data processing systems in the field and in headquarters units for control, distribution and prediction of requirements of critical medical supplies, such as blood, appears capable of programming with modification, so that it performs the blood inventory control and prediction operations required at field stations and headquarters units.

## Publications and/or Presentations:

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quires ABO blood grouping, serum c								
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ceptable system. A 15- channel autos	analy ze	r (Technicon)	will be placed	l in operation				
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### Detail Sheet # 1

## (U) Progress:

The Technicon Autoanalyzer system of blood grouping has been studied for accuracy and reproducibility. Certain parts have been found to be delicate in construction and require constant replacement. Many critical parts must be kept on hand for immediate replacement to keep the autoanalyzer in operation.

Cooperative studies with Dade Reagents, Inc., are continuing to produce standard reagents for the autoanalyzer that are licensed by the Division of Biologics Standards, National Institutes of Health.

In view of changes to AR 40-3, which now require ABO blood grouping, serum confirmation of the blood group by back typing with known A and B erythrocytes and determination of the Rh factor places a sense of urgency in developing accurate blood grouping automation, as well as training in the acceptable system.

Another automated system is being studied requiring joint studies and effort by the Blood Transfusion Division, USAMRL, and Berkeley Scientific Laboratories, Berkeley, California.

#### Publications and/or Presentations:

Camp, Frank R., Jr. and Charles E. Shields. Screening procedures employing semiautomated and fully automated technics. Presented (by Camp) at the 11th Congress of the International Society of Blood Transfusion, Sydney, Australia, 24-29 Aug 1966; USAMRL Report No. 707, 1966 (DDC AD No. 647538).

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## Detail Sheet # 2

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## Detail Sheet # 1

## (U) Progress:

The progress in FY 1967 on the hematological values, blood chemistry values and osmotic fragilities of Cercocebus spp. and Papio spp. of primates has been quite successful. The manuscript and bibliography of the results is in preparation for publication. As a result of this work, normal clinical laboratory values for this colony's primates are established and employed in routine and experimental evaluation of the animal committed to current project work.

Several areas of further interest have come to light as a result of this study. The comparison of primate leukocyte morphology to human leukocytes indicates that the primates commonly have hypersegmentation of polymorphonuclear (pmns) leukocytes. The significance of such findings is that hypersegmentation of pmns is a criteria in the diagnosis of vitamin B<sub>12</sub> and Folic Acid deficiencies in man. It is hoped that next year we will be able to determine if our primates either have a B<sub>12</sub> deficiency or a different control on leukocyte maturation which could normally lead to hypersegmentation. During the tabulation and comparison of the blood glucose levels of baboons, it was found that the animals were divided into those with extremely high blood glucose level or lower than normal levels. A study of the situation indicates that quite possibly there is an impaired glucose tolerance in primates. This, coupled with a pilot survey on glucose-6-phosphate dehydrogenase content of erythrocytes, further indicates that subhuman primates carbohydrate metabolism possibly follows different pathways and should be investigated for numerous reasons.

In the past year, this division has been able to produce an erythrocyte antiserum in rabbits against the cells of one baboon. The utilization of this antiserum in various immunohematologic techniques has enabled the separation of the rest of the baboon colony into two groups. Those groups having an erythrocyte factor similar to the baboon used to produce the antiserum or those lacking the factor. With this foundation and current investigations, it will be possible to iso-immunize baboons which are deficient in the erythrocyte factor and thereby increase the specificity of the antiserum. Concurrently with

A821 00 160 (cont)

## Detail Sheet # 2

the erythrocyte antiserum production has been the production of antiserum against whole baboon serum. It is now hoped that by fractionation of the baboon serum and further rabbit immunization with the components to be able to produce specific antiserum which will be of value in detection of possible incomplete blood groups of baboons.

With the assistance of Dr. Scott N. Swisher, Rochester Medical School, this laboratory's canine colony was typed for the four known canine blood groups (A<sub>2</sub>, A, C, and D). This information was subsequently utilized in the production of canine erythrocyte typing serum which is presently ready to be harvested and purified for distribution to individuals in need of this typing serum.

The results of all of these investigations have been twofold. First, it furthered our basic knowledge of this laboratory's animal colony and secondly, as this laboratory progresses in the study of transfusion complications, baseline data of this nature will be imperative for the utilization of laboratory animal models.

#### Publications and/or Presentations:

None.

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## Publications and/or Presentations:

None.

## Selected Bibliography:

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DeVenuto, F. Interaction of progesterone and aldosterone with red blood cells of the rat. Proc. Soc. Exper. Biol. and Med. 124: 478, 1967.

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## (U) Progress:

Hemolysates of stored red blood cells and the supernates obtained from high speed centrifugation of the hemolysates have been subjected to fractionation by anion-exchange chromatography utilizing a microgranular DEAE-cellulose column. The system employs gradient elution with a Tris-HCl buffer of decreasing pH and constant molarity. Modification of this gradient reported in studies on the heterogeneity of hemoglobin has reduced considerably the time required for elution of the constituents of the hemolysate with no apparent loss in resolution. At least two types of hemoglobin, as well as two non-hemoglobin proteins in low concentration, have been separated employing this chromatographic procedure. Small analytical columns have been used to date, and the procedure will be scaled up to preparative size to provide sufficient amounts of the separated components for more extensive analysis.

Concentration of the separated constituents has permitted preliminary examination with respect to electrophoretic mobility and homogeneity by means of disc gel electrophoresis.

Hemolysates prepared from red blood cells stored 0 to 28 days, with or without corticosterone added, have been subjected to column chromatography. No significant quantitative change attributable to time of storage has been observed in the hemolysates or their chromatographically separated constituents.

## Publications and/or Presentations:

None.

## Selected Bibliography:

Huisman, Titus H. J. and Andree M. Dozy. Studies on the heterogeneity of hemoglobin. IX. The use of tris-HCl buffers in the anion-exchange chromatography of hemoglobins. J. Chromatography, 19: 160, 1965.

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# Detail Sheet # 2

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Publications and/or Presentations:

None.

Selected Bibliography:

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#### Detail Sheet # 1

Publications and/or Presentations:

None.

## Selected Bibliography:

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Flick, D. F., J. B. Scott, and R. A. Hardin. Preliminary observations on the reversal of hypovolemia with intravenous fat emulsion. Proc. Soc. Exper. Biol. and Med. 124: 793, 1967.

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Work done under projects/tasks/work units of prior years; articles published in FY 1967.

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UNCLASSIFIED Security Classification					
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US Army Medical Research Laboratory	UNCLASSIFIED				
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Progress Report (1 July 1966 - 30 June	1967)				
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The progress during Fiscal Year 1967 and the current status of the various work units are reported herein. (U)

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